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Phenolics, Antiradical Assay and Cytotoxicity of Processed Mango *(Mangifera indica)* and Bush Mango (*Irvingia gabonensis*) Kernels

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ABSTRACT

Phenolic constituents (total phenols, flavonoids, tannins, and anthocyanins), comparative antiradical potency and cytotoxicity of processed mango *(Mangifera indica)* kernel (PMK), processed bush mango *(Irvingia gabonensis)* kernel (PBMK) and their mixture (MKK) at 50:50 were evaluated. Antiradical assay of the samples was conducted using three different methodologies (lipid peroxidation, nitric oxide and ferric reducing anti-oxidant power (FRAP)). The three samples contained negligible amounts of anthocyanins (<0.67 ng/g) compared with the other constituents (1.4 to 2.2 mg/g), largely of gallotannins and flavonoids. However, PBMK had the least flavonoid content, averaging 43%. Due to relative sensitivity of assay techniques to phenolic composition of sample extracts, results from FRAP assay favourably complemented those of another technique to give better reflection of sample's radical scavenging capacity. Mean inhibitory concentration (IC_{50}) values obtained, showed that the three samples had higher antioxidant capacity than reference quercetin. Similarly, the mean lethal concentration (LC_{50}) values obtained from cytotoxicity assay indicated that the phenolic acid and flavonoid content of the three processed kernel samples were more tolerable physiologically compared with reference dichromate. Both observations were statistically significant ($p < 0.05$). Processed kernels of mango *(Mangifera indica)* (PMK) and bush mango *(Irvingia gabonensis)* (PBMK), therefore, could find application as neutraceuticals and antimicrobials.

Keywords: Garlic acid, flavonoid, tannin, inhibitory concentration, cytotoxicity.

Introduction

Mango (*Mangifera indica*) fruit is relished globally for its edible mesocarp (Vandrendriessche, 1976), and the endocarp is discarded as waste especially in Africa. However, researches in Asia in the last three decades have shown the kernel portion of the endocarp to contain lipids of potential nutritional value, and industrial significance (Rukmini and Vijayaghavan, 1984). Similarly, another variety growing in wild tropical forests, popularly called 'bush mango' (*Irvingia gabonensis*) has been reported to have industrial significance and is largely used in traditional and modern medicine (Ude *et al.*, 2006). Methanolic extracts of *I. gabonensis* are used in the treatment of bacterial and fungal infections, similar to some other medicinal plants (Miliauskas *et al.*, 2004). Kernels of the fruits are used as soup thickener and the juice is used in wine production which is attributed to attenuating obesity in relation to treatment of type-II diabetes (Victor *et al.*, 2007).

Raw mango *(M. indica)* kernel is astringent and the report of Vaghasiya and Chanda (2010) stated that methanolic extract of the seed could be a source of natural antioxidants. The earlier works of Arogba (1999a, 1999b, 2001a, 1997, 2002) have shown the potential use of the kernel in human diet.

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Qualitative examination of phenolic compounds and the associated enzymes of the processed mango kernel (PMK) and bush mango kernel (PBMK) have been reported (Arogba *et al.*, 1998; Arogba, 1999c; Arogba and Ezeonye, 1999; Arogba, 2001b, 2001c). Recently, their antioxidant activity was also reported (Arogba and Omede, 2012).

Except for these reported studies, literature lacks details on the residual and relative concentrations of the phenolic components of these processed kernels (PMK, PBMK). The present study, therefore, quantitatively estimated the total phenols, flavonoids, tannins, and anthocyanins of PMK, PBMK and their mixture, and using more than one methodology to assess their in-vitro antiradical potency. Cytotoxicity study of PMK, PBMK and their mixture was also undertaken. The choice of solvent for extraction, and use of a single method to assess antioxidant activity has merits and limitations, more-so for the fact that radical scavenging activity of materials depends not only on the total phenolic content but also, on types and relative proportions of the individual components present (Hisalkar *et al.*, 2012). Combination of methods, therefore, was used to affirm total antioxidant capacity (TAC) of materials of interest.

Materials and Methods

Materials collection and handling

Fresh mango seeds (1 kg) of Ikanekpo variety were collected at Anyigba town (Kogi State, Nigeria) and Bush Mango (1 kg) was purchased from Anyigba market.

The methodology of Arogba (1997) was adopted to obtain the processed mango and bush mango kernels (PMK and PBMK respectively), each seed type (500 g) was shelled to free the kernels before soaking/sulphiting, blanching and drying. The PMK and PBMK were each milled into powdery form. The flour samples were used separately and in a blended form (50 : 50). For clarity, the adhering testa on the BMK was left intact.

Reagents

The main reagents included 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (SIGMA, Germany), vitamin C (Puritan's Pride Inc., Oakdale, USA), sodium metabisulphite, potassium dichromate, quercetin (SIGMA, Germany), methanol, sodium chloride.

Preparation of sample extracts

One gram (1 g) of sample was weighed into a beaker containing 50 ml of methanol, placed and shaken on orbital shaker for 2 h. After shaking, the supernatant was carefully decanted to form the stock solution from which, serial dilutions were made to obtain other required concentrations.

Determination of total phenolic content

The total phenolic content of the sample extract was determined using the Folin-Ciocalteau assay method of Singleton and Rossi (1965) as modified by Gulcin *et al.*, (2003). Distilled water (0.9 ml) was pipetted into 0.1 ml of 1 mg/ml of methanolic sample extract or reference in a test tube, followed by 0.2 ml of Folin reagent. The resulting mixture was then vortexed. After 5 min, 1 ml of 7% Na_2CO_3 solution was first added to the mixture, and further diluted to 2.5 ml before incubating for 90 min at ambient temperature. The absorbance was measured on a UV-visible spectrophotometer (Milton Roy Spectronic 601, USA) at 750 nm was then read against the reagent blank. Reference stock solution of gallic acid (1 mg/ml) was prepared, from which aliquots of 0.2, 0.4, 0.6, 0.8, and 1 ml were withdrawn and made up to 2 ml with methanol. The total phenolic content of the sample was then calculated, using the calibration curve drawn, and expressed as mg gallic acid equivalent (GAE)/g dry weight. Analysis was conducted in triplicate.

$C = c\ V/m$

where:

C = total content of phenolic compounds in gallic acid equivalent (GAE)

c = concentration of gallic acid extrapolated from the calibration curve, mg/ml

V = volume of methanolic sample extract (ml)

m = weight of the sample taken.

Determination of total flavonoid content

The determination of the total flavonoid content of the plant extract was based on the aluminium chloride colorimetric method of Zhilen *et al.* (1999) and modified by Miliauskas *et al.* (2004). Distilled water (0.4 ml) was added to 0.1 ml of sample extract or reference. This was followed by 0.1 ml of 5 % sodium nitrite. After 5 min of incubation, 0.1 ml of 10 % aluminium chloride and 0.2 ml of sodium hydroxide were added and the volume was made up to 2.5 ml with distilled water. The absorbance at 510 nm was measured against the blank.

A stock solution of quercetin (1 mg/ml) was prepared. Aliquots of 0.2, 0.4, 0.6, 0.8, and 1 ml were taken. Each volume was made up to 2 ml with distilled water and treated as described above, in order to prepare a calibration curve. The total flavonoid content of the sample was then calculated as shown in the equation below and expressed as mg quercetin equivalents per gram sample. The analysis was conducted in triplicate.

$X = q\ V/w$

Where X= total content of flavonoid compounds in quercetin equivalent

q = concentration of quercetin extrapolated from the reference curve

V = volume of extract (ml)

w = weight of the sample.

Determination of total anthocyanin content

Total anthocyanin content of the extract was determined by the pH differential method (Fuleki and Francis, 1968; Wrolstad, 1993). A pH 1.0 buffer solution was prepared by mixing 125 ml of 0.2 N KCl with 385 ml of 0.2 N HCl and 490 ml of distilled water. The pH of the buffer was adjusted to pH 1.0 with 0.2 N HCl.

A pH 4.5 buffer solution was also prepared by mixing 440 ml 0f 1.0 M sodium acetate with 200 ml of 1M HCl and 360 ml of distilled water. The pH of the solution was measured and adjusted to pH 4.5 with 1M HCl.

The sample extract (0.5 ml) was diluted to 12.5 ml in the pH 1.0 and 4.5 buffers and allowed to equilibrate in the dark for 2 h. The absorbance of the samples at 512 nm (A_{512} nm) and 700 nm (A_{700} nm) was measured. The difference in absorbance (ΔA) between the anthocyanin extract diluted in pH 1.0 and pH 4.5 buffers was calculated using the equation below:

$$\Delta A = (A_{512}\ pH1.0 - A_{700}\ nm\ pH1.0) - (A_{512}\ nm\ pH4.5 - A_{700}\ nm\ pH\ 4.5)$$

The A_{700} nm was employed to correct any background absorbance due to turbidity on the extracts. The anthocyanin content was expressed as mg cyanidin 3-glucoside per 100 g sample using a molar absorbance coefficient (ε) of 26900 $L^{-1}M^{-1}cm^{-1}$.

$$TACY = \frac{(\Delta A \times MW) \times DF \times 1000}{\varepsilon \times 0.1 \times 1}$$

Where:

TACY = Total anthocyanin expressed as mg cyanidin 3-glucoside/100 g of sample.

MW = molecular weight of cyanidin-3-glucoside (449.2 g/L)

DF = dilution factor to express the extracts on per gram of sample.

ε = molar absorption coefficient of cyanidin 3-glucoside (26900 $M^{-1}cm^{-1}$)

0.1 = is the conversion factor from per 1000 grams to 100 grams basis.

Determination of total tannin content

The vanillin method was used to determine total tannin content as described by Sundang *et al.,* (2012). Concentrations of 0, 5, 10, 20, 30, 40, 50 and 100 µg/ml of reference catechin were prepared to obtain a calibration curve, and 100 µg/ml of each sample was prepared to determine total tannin content in catechin equivalent. Samples or reference (1.0 ml) was mixed with 3.0 ml of 4 % (w/v) vanillin followed by addition of 1.5 ml 1 M HCl, then incubated in the dark for 5 min and the

absorbance measured at 500 nm using a UV-Vis spectrophotometer.

Lipid peroxidation assay

The anti-lipid peroxidative properties of the sample extract was determined using a modified thiobarbituric acid reactive species (TBARS) assay of Ohkowa *et al.* (1979) as described by Nabasree and Bratati (2004). In this assay, the end product of lipid peroxidation using liver homogenate as lipid-rich media (Ruberto *et al.*, 2000) was quantified by determining the formed adduct of malonyldialdehyde (MDA) reaction with thiobarbituric acid (TBA) under acidic condition. The pink colored product was measured at 532 nm on a spectrophotometer.

Procedure: To 0.5 ml of a 0.1 mg/ml liver homogenate was added 0.1 ml of varying concentrations of the extract (500, 250, 125, 62.5, 31.25 µg/ml) in a test tube followed by the addition of 1 ml distilled water. Then 50 µl of $FeSO_4$ (0.07 M) was added to the reaction mixture. The reaction mixture was vortexed and allowed to stand for 30 min at ambient temperature after which 1.5 ml of 20% (v/v) acetic acid and 1.5 ml of 0.8% (w/v) thiobarbituric acid in 1.1% (w/v) sodium dodecyl sulphate were added. The resulting mixture was then incubated in a water bath at 95^{0}C for 1 h. After cooling, 4.0 ml of butan-1-ol was added to each tube, shaken vigorously and centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured at 532 nm. The TBARS values were calculated using the extinction coefficient 1.56 x 10^{-5} M/cm. Inhibition of lipid peroxidation (%) by the extract was calculated using the formula

$(1 - E/C) \times 100$

where:
C = absorbance value of the fully oxidized control
E = absorbance in the presence of extract as $\{(A_{532} + TBA) - (A_{532} - TBA)\}$

Nitric oxide radical inhibition assay

Nitric oxide, generated from sodium nitroprusside in aqueous solution at physiological pH, interacts with oxygen to produce nitric ions which were measured by Griess reaction (Marocci *et al.*, 1994). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM) in phosphate buffered saline (PBS) and varying concentrations (500, 250, 125, 62.5, 31.25 µg/ml) of the extract were incubated in the dark at ambient temperature for 150 min. After incubation, 1.5 ml of the reaction mixture was removed and 1.5 ml of Griess reagent was then added. The absorbance of the chromophore formed was evaluated using spectrophotometer (Milton Roy Spectronic 601, USA) at 546 nm. Ascorbic acid was used as positive control and inhibition of nitric oxide radical (%) by the extract was calculated using the formula:

$[(1 - E/C)] \times 100$

where:
C = absorbance value of the fully oxidized control;
E = absorbance in the presence of extract.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay used antioxidants as reductants in a redox linked colorimetric method with absorbance measured using a spectrophotometer (Benzie and Strain, 1996), as described briefly by Hisalkar *et al.* (2012). In a semi-micro plastic cuvette, 50 µL aliquot of extract was mixed with 1.5 ml of FRAP reagent (25 ml of 3 M sodium acetate, pH 3.6; 2.5 ml of 0.01 M TPTZ {2, 4, 6-tri (2-pyridyl 1, 3, 5-triazine, 98 %(sigma-Aldrich)} in 0.04 M HCl; 2.5 ml of 0.02 M $FeCl_3.6H_2O$; preheated to 37^{o}C). Absorbance measurement was taken at 593 nm (A_{593}) exactly 10 minutes after mixing, and using 50 µL of water as the reference. Similarly, each varied concentration (0.2 to 1 mM) of reference $FeCl_3.6H_2O$ was mixed with 1.5 ml of FRAP reagent to prepare a calibration curve. All measurements were taken at ambient temperature with extracts protected from direct sunlight.

Brine shrimp cytotoxicity assay

Brine shrimp lethality bioassay was carried out to investigate the cytotoxicity of the PMK, PBMK and MKK. This was done using hatched brine shrimp *(Artemia salina)* larvae by the method of Meyer *et al.* (1982) in a vessel filled with simulated sterile artificial sea water (brine solution) by dissolving 16 g of sodium chloride analyte in 500 ml of distilled water.

Some 100 μg of brine shrimp eggs was added to small amount of the artificial sea water and incubated under bright light, connected to air-voltage pump that aerated the mixture. It was sealed and kept for 24 h to hatch. At the end of incubation, 10 brine shrimp larvae were counted and transferred to different vials using a Pasteur pipette and volume was made up to 10 ml with the artificial sea water.

Constant volume of 100 μl of each varied concentration (2000, 1000, 500, 250 and 125 μg/ml) of a sample type (PMK, PBMK, or MKK) or the reference potassium dichromate was added to the vials containing the shrimps. After 24 h, the dead larvae were counted for the determination of the percentage lethality.

Statistical analysis

The results were expressed as mean ± SEM using Graph Pad Prism Graphical-Statistical Package version 5. The difference between groups was analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's test at 5% level of significance ($p = 0.05$).

Results

The drying process took 24 h and the dry matter content was determined to be 95.2 ± 0.08 %, 93.9 ± 0.89 % and 92.1± 0.45 % for processed mango kernel (PMK), bush mango (PBMK) and their 50:50 mixture (MKK), respectively.

Phenolic composition

Three processed kernel powder samples (PMK, PBMK, MKK) were quantified for relative concentrations of four major phenolic components with antiradical properties, namely, total free phenols (p), flavonoids (f), tannins (t) and anthocyanins (a). Results in Table 1 show that the mixture (MKK) had twice, and about 25% more gallic acid equivalents than PBMK and PMK, respectively. The total flavonoid content of the samples expressed as quercetin equivalent per gram showed that PBMK had the least content, with 77 and 122% more in MKK and PMK, respectively. Comparatively, PMK and MKK had higher free phenolic and flavonoid content than PBMK. The lower flavonoid content betwen the pair was about 115% and within the PBMK sample by 50%. These observations were statistically significant at $p = 5\%$, as was similarly observed within MKK with about 60% lower flavonoid content. The tannin content of PMK and PBMK samples were similar, with no significant ($p > 0.05$) difference but the mixture (MKK) had relatively higher content and was significantly different ($p < 0.05$).

The concentration of anthocyanin in the samples was in the range of 0.14 – 0.67 nanogram equivalents per gram sample compared with the milligram quantities of the other components discussed.

The relationship between any two phenolic components was calculated to give correlation coefficients, r, as p/t 0.9538, f/t 0.7185, p/f 0.4764, p/a 0.4074, f/a 0.3790, and a/t -0.3714.

Antiradical assays

The results of one of the three antiradical assays carried out on the different test fractions are shown in Table 2. In the lipid peroxidation assay, MKK showed the highest scavenging activity as evidenced by the IC_{50} values of 123.65 ± 1.78 μg/ml. However, IC_{50} values from 123 to 157 μg/ml for MKK, PMK, and reference vitamin C, were not significantly different ($p > 0.05$). Quercetin, which had the highest percentage inhibition (80 %) surprisingly, showed the next higher level of activity with IC_{50} of 214.14 μg/ml, and was significantly different ($p < 0.05$) from the lowest activity value of 375.38 ±1.28 μg/ml for PBMK.

From nitric oxide inhibition assay (Table 3), PBMK and vitamin C had the highest radical scavenging activity, followed by MKK, and (PMK, Quercetin) in that order. Their IC_{50} values were significantly different ($p < 0.05$). As in the lipid peroxidation assay, quercetin also had the highest percentage inhibition of 83.19±1.36 % with IC_{50} of 177.17±1.33 μg/ml, but exhibited the least antiradical activity using this methodology.

The FRAP assay result (Table 4) also showed a concentration-dependent change when the FRAP values of the samples were determined. Ferric-reducing ability of the samples were expressed in mmol Fe^{2+}/L. The highest radical scavenging activity expressed by IC_{50} in mg/ml, was exhibited by MKK and followed by PMK. PBMK had the next and similar level of activity with vitamin C. A cross-reference with IC_{50} of rutin (Hisalkar *et al.,* 2012) of similar structure to quercetin, would rate quercetin lowest in activity using FRAP methodology.

Cytotoxicity assay

The death-rate of brine shrimp was concentration-dependent (Table 5). While about 2 mg/ml dose of reference potassium dichromate killed all the brine shrimp larvae, 15 to 20 % still survived with similar dosage of the three processed kernel extracts. From regression analysis, the mean lethal concentrations (LCs_{50}) of the kernels differed significantly ($p < 0.05$) from that of the reference dichromate. PBMK had the highest lethal concentration (LC_{50}) of 549 μg/ml, and not significantly different ($p > 0.05$) from those of PMK, and MKK. The reference dichromate had LC_{50} of 342 μg/ml.

Discussion

Fruits, vegetables and oil seeds have increasingly been recognized as natural sources of phenolic antioxidants (Shahidi, 1997; Nack and Shahidi, 1989), and proved to be safer even in chronic administration (Adaramoye *et al.,* 2008; Oyaizu, 1986) of illnesses. Several epidemiological studies also, have shown that intake of exogenous antioxidants is effective in the prevention of a number of human diseases which have been complicated by oxidative stress (Valentao *et al.,* 2002).

The present study became necessary to quantify the phenolic content of the processed kernels and assess their antiradical potential for intended application in food systems. Arogba (1999c) had employed chromatographic technique to identify tannins of mango *(M. indica*) kernel. It was reported that before acid hydrolysis, the free (unbound) phenolic compounds were tannic acid, gallic acid, and epicatechin in the ratio 17:10:1, while hydrolysis of the condensed tannins revealed the presence of ellagic acid, gallocatechin, and an acylated cyanidin in the ratio 11:7:5. It was obvious, therefore, to note the large presence of simple phenolic acids, and flavonoids. Results obtained in Table 1 confirm the dominance of gallic acid and quercetin equivalents in the processed mango (*M. indica*) and bush mango *(I. gabonensis)* kernels (PMK, PBMK) and their 50:50 mixture (MKK).

Table 1: Concentration of some phenolic constituents

Constituents	PMK	PBMK	MKK
Total phenol (mg GAE/g dry wt)	1.67 ± 0.13^{b}	1.15 ± 0.03^{a}	2.19 ± 0.08^{c}
Total flavonoids (mg QUE/g) dry wt)	1.64 ± 1.02^{b}	0.77 ± 0.23^{a}	1.38 ± 0.18^{c}
Anthocyanin (ng cyanidin chloride/g dry wt)	0.14 ± 0.07^{a}	0.67 ± 0.09^{c}	0.33 ± 0.02^{b}
Tannins (mg catechin/g dry wt)	1.35 ± 0.35^{a}	1.25 ± 0.81^{a}	1.69 ± 0.98^{b}

All values are expressed as mean ± SEM (n=3). Values with different letters on the same row are significantly different ($p < 0.05$).

However, the flavonoid content of PBMK was significantly ($p < 0.05$) lower than the other pair, with an implication in understanding the relative concentrations of these phenolic components in the kernel-types. The computed correlation coefficients, r, between any two components gave a good guide. The negative r-value of -0.3714 between anthocyanin and tannin supports possible trace concentration of glycosidic linkages in the tannin composition of these kernels. Rather, the high molecular weight compounds of the kernel samples appeared to be polymeric forms of their phenolic acids and flavonoids, as indicated by r-values of 0.9538 and 0.7185 between free phenols/tannins, and flavonoids/tannins, respectively.

Processing fresh PMK and PBMK into flour resulted in dry weight matter content comparable with the previous work of Arogba (1997). During processing, Arogba (1997) had indicated leaching of water-soluble reducing substances such as polyphenols that impart astringent property to the kernels, and leaving residual amounts as preservatives in the processed flour to be used as principal ingredient for preparing edible products (Arogba, 1999a, 1999b, 2001a, 2002) such as biscuit, bread and pastries. The phenolic content of the processed kernels strongly accounts for the results of antiradical activity presented in Tables 2, 3, and 4. More critically however, is the fact that antiradical potency of a material depends on phenolic composition, relative concentrations of the components, and the available reducing groups (Khan *et al.,* 2012). Antiradical activity has been correlated directly with antioxidant activity, where available hydroxyl groups provide hydrogen radicals that prevent peroxidation or participate in the termination of radical chain reactions (Nana Fernand *et al.,* 2012; Re *et al*., 1999). Antioxidants play vital preventive role in degenerative illnesses caused by oxidative stress. In living organisms, complex natural substances (glutathione, vitamins C and E) and enzymes (superoxide dismutase, glutathione peroxidase, catalase), in addition to foods of plant origin, provide total antioxidant capacity for the system (Stintzing and Carle, 2004; Duvivier *et al*., 2010; Hisalkar *et al.,* 2012).

Various organic solvents have been employed by researchers (Das and De, 2011) to extract active antiradical constituents from materials, but methanol was used in this study, and IC_{50} computed as the index of antioxidant activity. From lipid peroxidation, and FRAP assays (Tables 2 and 4), PMK and MKK showed similar antioxidant activity, while PBMK activity correlated more with that of reference vitamin C when nitric oxide, and FRAP assay results (Tables 3 and 4) were compared.

Table 2: Lipid peroxidation assay

Concentration µg/ml	PMK (%)	PBMK (%)	MKK (%)	Vitamin C (%)	Quercetin (%)
500	62.08 ± 2.31	52.43 ± 1.97	68.62 ± 3.02	72.20 ± 0.94	80.33 ± 1.11
250	55.11 ± 0.78	47.11 ± 0.89	57.97 ± 1.23	61.50 ± 1.26	67.03 ± 1.20
125	51.75 ± 1.08	47.87 ± 1.45	51.77 ± 0.69	53.16 ± 2.07	50.04 ± 0.89
62.5	49.81 ± 2.14	43.22 ± 1.12	46.87 ± 1.81	30.60 ± 3.01	26.23 ± 0.34
31.25	39.17 ± 0.78	36.25 ± 0.95	43.06 ± 2.11	9.88 ± 0.41	13.07 ± 1.36
IC_{50}	156.57 ± 1.42^{a}	375.38 ± 1.28^{c}	123.65 ± 1.78^{a}	110.49 ± 1.54^{a}	214.14 ± 0.98^{b}

%: percentage inhibition.

All values are expressed as mean ± SEM (n = 3). Values with different letters on the same row are significantly different ($p < 0.05$).

Except for PBMK activity in lipid peroxidation assay, the three assays affirmed that methanolic extracts of the processed kernels contained more potent antioxidants than the reference quercetin ($p < 0.05$). The observation agrees with the recent report by Arogba and Omede (2012) when the processed kernels were assayed using only DPPH (2, 2-diphenyl-1-picrylhydrazyl hydrate) technique. From the view-point of composition, it could be inferred that high number of unbound reducing groups was available in the processed kernels for quenching radical reactions. Furthermore, the results suggested that both the pH and thermal conditions of the processing could have hydrolysed polymerised gallic acid or flavonoid equivalents present as tannins in these kernels. The order of antioxidant activity based on IC_{50}, was MKK>PMK >PBMK (Tables 2, 3 and 4) ($p < 0.05$).

Table 3: Nitric oxide assay

Concentration (µg/ml)	PMK (%)	PBMK (%)	MKK (%)	Vitamin C (%)	Quercetin (%)
500	69.59 ± 1.72	71.65 ± 3.11	75.26 ± 0.34	78.25 ± 1.90	83.19 ± 1.36
250	67.89 ± 0.57	67.34 ± 0.13	68.19 ± 0.22	65.50 ± 0.86	72.13 ± 1.85
125	56.34 ± 0.88	62.87 ± 0.89	52.43 ± 1.66	49.19 ± 0.78	54.54 ± 2.36
62.5	39.98 ± 1.23	40.65 ± 1.60	43.78 ± 1.38	32.56 ± 2.07	31.08 ± 0.71
31.25	27.39 ± 2.43	37.23 ± 1.49	32.84 ± 2.04	20.79 ± 0.69	19.81 ± 0.36
IC_{50}	164.63 ± 1.37^{c}	106.12 ± 1.44^{a}	140.00 ± 2.82^{b}	103.16 ± 1.26^{a}	177.17 ± 1.33^{c}

%: percentage inhibition.
All values are expressed as mean ± SEM (n=3). Values with different letters on the same row are significantly different ($p < 0.05$).

Table 4: Antioxidant activity using FRAP assay

Concentration (µg/ml)	PMK (mM Fe^{2+})	PBMK (mM Fe^{2+})	MKK (mM Fe^{2+})
500	1.09 ± 0.16	0.89 ± 0.18	1.75 ± 0.14
250	0.94 ± 0.08	0.78 ± 0.16	0.90 ± 0.08
125	0.81 ± 0.16	0.60 ± 0.13	0.79 ± 0.18
62.5	0.70 ± 0.04	0.59 ± 0.09	0.56 ± 0.28
31.25	0.49 ± 0.16	0.51 ± 0.33	0.42 ± 0.12
IC_{50} (mg/ml)	44.92^{b}	65.43^{c}	34.80^{a}
IC_{50} (mg/ml) Ascorbic acid	65.0^{c} (Hisalkar *et al.*, 2012)		
IC_{50} (mg/ml) Rutin	75.3^{d} (Hisalkar *et al.*, 2012)		

Values are expressed as mean ± SEM (n = 3). Values with different letters on the same row are significantly different ($p < 0.05$).

The *in vitro* lethality assay of brine shrimp *(Artemia salina)* was conducted to detect cell toxicity. Shrimps have always been considered as a preliminary tool for toxicity assessment from pharmacological point of view. The test was able to show that the three forms of the processed kernels had higher LCs_{50} than the reference potassium dichromate used (Table 5), implying the bio-safety of the samples. For the possible reason of similarity in phenolic composition, the samples had comparable LCs_{50} but significantly different ($p < 0.05$) from LC_{50} of the reference dichromate. Using regression analysis, the brine shrimp lethality test was observed to show linear proportionality between percentage lethality and concentrations of the processed kernels and $K_2Cr_2O_7$ used as reference. The results, in similarity with those of Omale *et al.* (2008), could imply that the samples had tolerable range of reducing substances, largely gallotannins, which would confer their *in-vivo* application in treatment of weight loss, lowering cholesterol levels and control of diabetes.

Table 5: Brine shrimp lethality assay

Concentration (µg/ml)	PMK (%)	PBMK (%)	MKK (%)	$K_2Cr_2O_7$ (%)
2000	85	80	80	100
1000	70	75	65	80
500	60	60	50	70
250	50	45	45	65
125	20	20	40	10
LC_{50}	521.21^{bc}	549.05^{c}	491.83^{b}	342.42^{a}

%: percentage inhibition.

All values are expressed as mean (n = 3). Values with different letters on the same row are significantly different ($p < 0.05$).

Conclusion

The results of this study, using the quantitative analysis, the *in vitro* antiradical, and *in vivo* lethality assays, have shown that processed kernels of mango *(Mangifera indica)* and bush mango *(Irvingia gabonensis)* contain significant amounts of gallotannins with high antioxidant capacity, which are at physiological tolerable range (LC_{50} range: 490 – 550 ppm of methanolic extract). However, IC_{50} and LC_{50} determinations would influence their applications as nutraceuticals and antimicrobials.

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